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ABSTRACT: Surveys for disease agents were conducted in introduced free-ranging elk (*Cervus elaphus nelsoni*) in Arkansas and Kentucky. Elk had been captured in Colorado and Nebraska and released in Arkansas during 1981–1985. From 1997 through 2002 elk were captured in Arizona, Kansas, North Dakota, New Mexico, Oregon, and Utah and released in southeastern Kentucky. Specimens were collected from 170 hunter-killed elk in Arkansas during 1998–2006, and 44 elk in Kentucky during 2001–2004. Significant findings included isolation of *Mycobacterium avium paratuberculosis* from one elk in Kentucky and evidence of previous or current infections by *Parelaphostrongylus tenuis* in several animals in Arkansas. Serological tests provided evidence of previous infection by epizootic hemorrhagic disease virus, bluetongue virus, bovine viral diarrhea virus, infectious bovine rhinotracheitis virus, parainfluenza-3 virus, and multiple serovars of *Leptospira interrogans*. *Mycobacterium bovis*, *Brucella abortus*, chronic wasting disease (CWD), and hemoparasites such as *Anaplasma* spp. were not detected. Results from elk obtained through these surveys were consistent with exposure to disease agents endemic in livestock and wildlife in Arkansas and Kentucky.

Key words: *Cervus elaphus*, elk, *Mycobacterium avium* subs. *paratuberculosis, Parelaphostrongylus tenuis*, survey.

INTRODUCTION

In 1981, the Arkansas Game and Fish Commission (AGFC), in cooperation with private citizens, began a program for restoration of elk (*Cervus elaphus*) in the Ozark Mountains of northwest Arkansas. From 1981 to 1985, 112 Rocky Mountain elk (*C. elaphus nelsoni*) were captured in southwest Colorado (Ouray, Delta, and Park Counties) and western Nebraska (Dawes County) and released in Newton County, Arkansas, near the Buffalo National River. Blood was collected from at least 21 of the elk captured in Colorado. Blood tests were negative for antibodies against *Brucella abortus* and *Leptospira interrogans*, but details of the tests used are not available (V. Graham, pers. comm.). No other procedures or treatments were used to assess the risk of releasing ectoparasites or infectious agents with the elk (Cartwright, 1995).

Since 1985, the Arkansas elk have reproduced and the herd has grown to an estimated 500 animals occupying a range of approximately 155,400 ha in portions of six counties. In 1998, the first elk hunts on public and private land in Arkansas were offered as part of the state's long-range management plan (Cartwright et al., 2001).

From 1997 to 2002, the Kentucky Department of Fish and Wildlife Resources (KDFWR), in cooperation with the Rocky Mountain Elk Foundation and the University of Kentucky, released 1,551 Rocky Mountain elk in the Cumberland Plateau physiographic region of southeastern Kentucky. Elk were captured in Arizona, Kansas, North Dakota, New Mexico, Oregon, and Utah. Prior to transport, state fish and wildlife agency personnel treated all elk with an anthelmintic (Dectomax®, Pfizer Animal Health, Exton, Pennsylvania; Ivomec®, Merial,

Duluth, Georgia, USA). Elk were tested for bovine tuberculosis, brucellosis (*Brucella abortus*), anaplasmosis (*Anaplasma* spp.), bluetongue (BT), vesicular stomatitis–New Jersey (VSNJ), VS-Indiana (VSI), and Johne's disease (*Mycobacterium paratuberculosis*; Kentucky Department of Fish and Wildlife Resources, unpubl. data). Elk were released on reclaimed coal mining land at eight sites within 14 counties designated as the "restoration zone." The current 16-county elk restoration zone encompasses more than 1.08 million ha and the current estimated population is 11,380 (postcalving 2009). Elk hunts were offered beginning in 2001.

The objective of our survey was to sample elk harvested during public hunts in Arkansas and Kentucky opportunistically to look for evidence of infection by selected disease agents. Organisms targeted were those thought to be of potential risk for introduction into the southeastern USA, and/or known to have pathologic consequences for free-ranging and captive wildlife and livestock (Corn and Nettles, 2001).

MATERIALS AND METHODS

During 1998–2006, 170 free-ranging elk were surveyed for selected disease agents in the Ozark Mountains in northwest Arkansas. Annual collections ranged from 9 to 29 animals representing roughly 2–6% of the population. These surveys represent a subset of the population harvested by elk hunters in Boone, Carroll, Newton, and Searcy counties during elk hunts held in September and December of each year. Specimens were collected by the Southeastern Cooperative Wildlife Disease Study and Arkansas Game and Fish Commission at either the kill site or at a check station. During 1998–1999 all internal organs were examined and tissues and blood were collected. During 2000–2006 only blood and brain stem at the level of the obex were collected.

Elk were sampled in eastern Kentucky during 2001–2004 similarly to the procedures used in Arkansas. Annual collections ranged from 9 to 13 animals, representing roughly 0.3–0.5% of the population. Specimens were collected from 44 elk harvested in Knott, Perry, and Breathitt counties near Hazard, Kentucky, USA. During the 2001–2003 sampling periods, all internal organs were examined and tissues and blood were collected. During 2004 tissues were collected only for *Mycobacterium avium* subspecies *paratuberculosis* (*Mptb*) culture.

Tissue specimens collected for microscopic examination were fixed in 10% neutral buffered formalin. Tissues collected in Arkansas during 1998–1999 and in Kentucky during 2001–2003 included cerebrum, cerebellum, brain stem, cervical spinal cord, retropharyngeal, and submandibular lymph nodes, heart, lung, liver, spleen, kidney, gonad, rumen, abomasum, and ileocecal, and mesenteric lymph nodes. Following formalin fixation, tissues were embedded in paraffin, sectioned at 3–4 μm, stained with hematoxylin and eosin (H & E), and examined by light microscopy. All sections of ileum and lymph node containing inflammatory lesions also were stained with Ziehl-Neelson acid-fast stain. Brain-stem tissue at the level of the obex collected during 2000–2006 was examined for spongiform lesions and/or accumulation of prion proteins (PrP) associated with chronic wasting disease (CWD) by immunohistochemistry (Spraker et al., 2002).

Serologic surveys for *Mptb* infection were based on the enzyme-linked immunosorbent assay (ELISA; IDEXX, kit as formulated in 2004, Portland, Maine, USA) and agar gel immunodiffusion (AGID) assay (ImmuCell, Portland, Maine, USA) following the manufacturer's instructions. Surveys also included radiometric culture of feces and lymph nodes (ileocecal and mesenteric). Samples were shipped on ice packs overnight to the Johne's Information Center (School of Veterinary Medicine, University of Wisconsin, Madison, Wisconsin, USA) for isolation of *Mptb* via the radiometric (BACTEC) culture method as described by Collins et al. (1990).

Between May 1997 and December 1999, five elk from the Buffalo National River area (Newton, Searcy, and Carroll counties, Arkansas) were observed to be emaciated and displaying a variety of neurologic signs. Clinical signs ranged from circling and ataxia to profound depression or loss of awareness of surroundings and the presence of humans. The elk were dispatched by thoracic gunshot and field necropsies were performed. Tissues were collected and processed for histopathology, as described, and fresh tissues (brain, lung, liver, spleen, ileocecal lymph node, and ileum) were collected and frozen at −70 C.

Blood was obtained by heart puncture or by

collection of free blood from the thoracic cavity. Blood was stored at room temperature or on wet ice for up to 24 hr. Serum was removed from the collection tubes after the blood had clotted or after centrifugation. Serum was stored on dry ice or in a freezer at −20 C. Serologic testing was conducted at the Athens Diagnostic Laboratory (College of Veterinary Medicine, University of Georgia, Athens, Georgia, USA) and the Johne's Information Center. The serologic tests and interpretation criteria (minimum cutoff for positives) were as follows: *B. abortus*, card test, positive or negative; bluetongue (BT) virus, agar gel immunodiffusion (AGID), positive or negative; bovine viral diarrhea (BVD) virus type 1, serum neutralization, 1:64; epizootic hemorrhagic disease (EHD) virus, AGID, positive or negative; infectious bovine rhinotracheitis (IBR) virus, serum neutralization, 1:16; parainfluenza 3 (PI3) virus, serum neutralization, 1:64; *Mptb*, ELISA, sample-to-positive (S/P) ratio of 0.25 using kit bovine sera controls with *C. elaphus* positive and negative controls on each plate and AGID, positive or negative; and *Leptospira interrogans* serovars *bratislava*, *canicola*, *grippotyphosa*, *hardjo*, *icterohaemorrhagiae*, and *pomona*, serum neutralization, 1:100. Whole blood was collected in tubes with ethylenediamine tetra-acetic acid (EDTA), stored at room temperature or on wet ice for up to 24 hr, and used to make blood smears.

RESULTS

No histologic evidence of *Mptb* (AR: 0/31; KY: 0/10), *M. bovis* (AR: 0/31; KY: 0/27), CWD (AR: 0/10; KY: 0/17), and *B. abortus* (AR: 0/18; KY: 0/12), nor blood parasites, i.e., *Anaplasma* spp., microfilaria, and trypanosomes (AR: 0/17; KY: 0/10) was found. The CWD prion protein was not apparent in obex sections collected from elk (AR: 0/74; KY: 0/17) that were tested by immunohistochemical staining. Histologic examination did reveal evidence of previous or current infection by *Parelaphostrongylus tenuis* (AR: 4/28, 14.3%; KY: 0/18): mild, multifocal lymphocytic/plasmacytic leptomeningitis with occasional eosinophils suggestive of previous *P. tenuis* infection was found in two Arkansas elk in September 1998; multifocal lymphocytic/plasmacytic perivascular cuffs and meningoencephalitis were seen in two Arkansas elk in December 1998, with multiple nematode larvae morphologically consistent with *P. tenuis* in the meninges over the cerebral cortex in one of these animals. Multifocal protozoal cysts (*Sarcocystis* sp.) were observed in the myocardium of elk (AR: 25/32, 78.1%; KY: 15/22 68.2%).

Serologic tests provided evidence of previous infection by EHD, BT, BVD, IBR, PI3, and *L. interrogans* serovars *bratislava*, *canicola*, *grippotyphosa*, *hardjo*, *icterohaemorrhagiae* and *pomona* (Tables 1 and 2). Serologic evidence of exposure to *B. abortus* was not found (Tables 1 and 2).

No isolates of *Mptb* were obtained from mesenteric lymph nodes (0/25) or feces (0/32) from elk in Arkansas in 1998–1999. Serum ELISA S/P results from serum from 5/81 Arkansas elk killed during 1998–1999 were above the manufacturer's S/P 0.25 cutoff for cattle; four were between 0.35 and 0.45, while the fifth's S/P was 1.65. All AGID results for these sera were negative. In Kentucky during 2001–2004, *Mptb* was isolated from the lymph node of one elk (1/44; 2.3%). No isolation of *Mptb* was made from Kentucky elk feces (0/32) and all sera ELISA and AGID were negative.

Five additional elk (clinical cases) euthanized and examined in the field were between 9 mo and 2.5 yr of age based on tooth eruption (Dimmick and Pelton, 1996). Four of five (80%) were in poor nutritional condition or emaciated; one elk still carried adequate adipose stores. One–three thin, threadlike nematodes were observed in the leptomeninges over the cerebellum in two (40%) of the elk. Microscopic examination of sections of brain from all five elk revealed inflammatory lesions in the leptomeninges, neuropil of the cerebellum and cerebrum, or both, with mixed perivascular infiltrates of lymphocytes, plasma cells, variable numbers of hemosiderin-laden macrophages, and frequent eosinophils. In three of five

TABLE 1. The proportion of collected Arkansas elk testing positive for antibodies to *Brucella abortus*, *Leptospira interrogans* serovars, BT, BVD, EHD, IBR, and PI3 during 1998–2006.[a]

Agent	1998	1999	2000	2001	2002	2003	2004	2005	2006	Total	%
Brucella abortus	0/25[b]	0/29	0/19	0/26	0/14	0/9	0/19	0/11	0/18	0/170	0
Leptospira interrogans serovar											
bratislava	1/25	3/29	0/19	0/26	0/14	0/9	6/19	1/11	6/18	17/170	10
canicola	1/25	0/29	0/19	0/26	0/14	0/9	0/19	0/11	0/18	1/170	0.6
grippotyphosa	4/25	0/29	1/19	0/26	1/14	1/9	0/19	0/11	0/18	7/170	4.1
hardjo	0/25	0/29	0/19	2/26	0/14	0/9	0/19	0/11	0/18	0/170	0
icterohaemorrhagiae	6/25	0/29	1/19	0/26	0/14	0/9	0/19	0/11	2/18	9/170	5.3
pomona	0/25	1/29	0/19	0/26	0/14	0/9	0/19	0/11	0/18	1/170	0.6
BT	2/25	0/29	0/19	12/26	1/14	2/9	0/19	0/11	5/18	22/170	12.9
BVD	1/25	1/29	0/19	2/26	0/14	1/9	1/19	0/11	0/18	6/170	3.5
EHD	6/25	2/29	1/19	11/26	1/14	2/9	4/19	0/11	7/18	34/170	20
IBR	4/25	0/29	0/19	2/26	0/14	1/9	0/19	0/11	0/18	7/170	4.1
PI3	2/25	0/29	0/19	0/26	1/14	0/9	4/19	0/11	0/18	7/170	4.1

[a] BT = bluetongue virus, BVD = bovine viral diarrhea virus, EHD = epizootic hemorrhagic disease virus, IBR = infectious bovine rhinotracheitis virus, and PI3 = parainfluenza-3 virus.

[b] Number positive/number tested.

elk (60%), there were multifocal areas of malacia in the neuropil of the brainstem (medulla oblongata and pons), cerebellum, thalamus, and/or cerebral cortex, containing sheets of mononuclear phagocytic (gitter) cells mixed with lymphocytes and eosinophils. In three of five elk (60%) the leptomeninges over the cerebellum or the neuropil of the cerebellum and/or cerebral cortex contained sections of adult nematodes morphologically consistent with *P. tenuis* (Chitwood and Lichtenfels, 1972) or contained sections of embryonated nematode eggs and free nematode

TABLE 2. The proportion of collected Kentucky elk testing positive for antibodies to *Brucella abortus*, *Leptospira interrogans* serovars, BT, BVD, EHD, IBR, and PI3 during 2001–2003.[a]

Agent	2001	2002	2003	Total	%
Brucella abortus	0/9[b]	0/12	0/10	0/31	0
Leptospira interrogans serovar					
bratislava	0/9	0/12	1/10	1/31	3.2
canicola	0/9	0/12	0/10	0/31	0
grippotyphosa	1/9	7/12	0/10	8/31	25.8
hardjo	1/9	0/12	0/10	1/31	3.2
icterohaemorrhagiae	0/9	1/12	0/10	1/31	3.2
pomona	0/9	0/12	1/10	1/31	3.2
BT	0/9	0/12	0/10	0/31	0
BVD	0/9	0/12	0/10	0/31	0
EHD	0/9	1/12	0/10	1/31	3.2
IBR	0/9	0/12	6/10	6/31	19.3
PI3	1/9	1/12	2/10	4/31	12.9

[a] BT = bluetongue virus, BVD = bovine viral diarrhea virus, EHD = epizootic hemorrhagic disease virus, IBR = infectious bovine rhinotracheitis virus, and PI3 = parainfluenza-3 virus.

[b] Number positive/number tested.

larvae. The CWD prion protein was not found in any of the specimens from these five elk.

DISCUSSION

Elk examined during public hunts in Arkansas and Kentucky appeared to be in good health, but clinical parelaphostrongylosis was found in Arkansas elk with neurologic signs. *Parelaphostrongylus tenuis* is harbored without apparent clinical signs by white-tailed deer throughout the southeastern USA, with the exception of the lower coastal plain (Anderson and Prestwood, 1981). This nematode is a potential threat to elk introduced into the southeast because infection can result in severe neurologic damage when the nematodes migrate through the spinal cord and brain (Samuel et al., 1992). The survey results and clinical cases we report include both clinically ill elk and healthy elk with subclinical or apparently resolved infections. Clinical parelaphostrongylosis was the most frequently encountered disease condition in this study, but the presence of subclinical and resolved infections confirms that not all meningeal worm infections proceed to or continuously cause clinical disease (Woolf et al., 1977). All five of the clinically affected elk had lesions in the brain and nematodes consistent with *P. tenuis* were observed within these lesions in three of five animals. All five elk were 2.5 yr of age or younger. In a study of meningeal worm infections in the reintroduced elk herd in Kentucky, Larkin et al. (2003) found that 73% of deaths attributable to *P. tenuis* occurred in elk less than 3 yr old. These studies provide evidence either that young elk with clinical disease die before reaching 3 yr of age or that animals surviving to a more mature age may be more resistant to development of significant nervous system lesions and resultant clinical signs.

We did not find evidence of *Mptb* infection in the Arkansas elk; the negative culture and histopathology findings suggest that the five sera that reacted to ELISA likely were false positives. This interpretation is strengthened by the uniformly negative AGID results of these sera, as AGID is known to have a lower sensitivity yet higher specificity than ELISA in some species (Hope et al., 2000). Davidson et al. (2004) report similar *Mptb* ELISA and AGID serologic status in a few culture-negative white-tailed deer in the southeastern USA, suggesting ELISA reaction to a non-*Mptb* organism. Despite our negative test results, however, it is not possible to state with certainty that the Arkansas elk population is free of *Mptb* infection given the low sensitivity of postmortem analyses for detection of *Mptb* in clinically normal animals.

In Kentucky, the single isolation of *Mptb* (from one lymph node) indicates the presence of the organism locally, but no conclusions can be drawn about its prevalence or impact on the health of the elk in that region (Sleeman et al., 2009). Natural infections of free-ranging cervids with *Mptb* are rare, and recent isolated cases are suggestive of spillover from nearby domestic animals (Davidson et al., 2004; Sleeman et al., 2009). Natural infection in wild elk has only been reported in two herds of tule elk (*Cervus elaphus nannodes*) in California (Jessup et al., 1981; Cook et al., 1997; Manning et al., 2003; Crawford et al., 2006).

Serologic evidence indicated exposure of less than 10% of the tested elk to the agents of BVD, IBR, PI3, and six serovars of *L. interrogans*. Antibody prevalence against BT and EHD viruses ranged from 0% to 46% and 0% to 42%, respectively, with peak prevalence observed during 2001. Hemorrhagic disease in white-tailed deer, caused by either BT virus or EHD viruses, occurs regularly in the southeastern USA (Nettles et al., 1992). Clinical illness due to BT and EHD has not been reported in free-ranging elk (Howerth et al., 2001); however, clinical neurologic

disease associated with EHD virus was seen in captive and free-ranging elk in Colorado, Montana, and Wyoming during 2001–2002 (Cornish, unpubl. data).

We did not find evidence of selected disease agents of concern, including CWD prions, *B. abortus* and *Mycobacterium bovis,* but because of the limited number of animals available for necropsy, we are unable to confirm the absence of these diseases within the Arkansas and Kentucky populations. Statistically, the theoretical upper limits of prevalence of these diseases based on our small sample size can be estimated as follows (assuming a 95% confidence level and 100% sensitivity and specificity for each test; Corn and Nettles, 1995): CWD ≤4% in Arkansas and ≤17% in Kentucky; *B. abortus*≤1.7% Arkansas, ≤10% Kentucky; *M. bovis*≤10% AR, ≤11% Kentucky. Limitations in the sensitivity and specificity of each assay curb the level of confidence of these prevalence estimates, but the extent of these limitations is difficult to quantify as most assays have not been validated in wildlife.

Despite the small sample size, we believe there is a low likelihood that these three disease agents were introduced or are currently present in the Arkansas and Kentucky elk herds. This interpretation is based on the absence of these disease agents in all animals tested in this survey coupled with that fact that CWD, *B. abortus*, and *M. bovis* were all unreported from the regions of origin of the translocated elk at the time the translocations occurred (Clifton-Hadley et al., 2001; Godfroid, 2002; US Department of Agriculture, 2009).

During the 1980s, CWD was endemic only in the north-central regions of Colorado, and testing for evidence of CWD in the late 1990s in the vicinity of capture locations revealed no evidence of the disease (Miller et al., 2000; Graham, pers. comm.). In Nebraska, CWD was first found in captive elk in 1998, at least 13 yr after elk were translocated from that state to Arkansas (Miller and Fischer, 2000). At the time of elk translocation to Kentucky, CWD had not been reported in Arizona, Kansas, North Dakota, or Oregon (US Department of Agriculture, 2009). Chronic wasting disease–positive mule deer were first found in south-central New Mexico and eastern Utah in 2002 around the time of the last elk translocation from these states; however, translocation sites were more than 100 miles from the locations of positive deer (New Mexico Wildlife, 2004; Utah Division of Wildlife Resources, 2006). Chronic wasting disease currently occurs in free-ranging elk in northwest and north-central Colorado, southeast, south-central and northeast Wyoming (Spraker et al., 1997; Williams and Young, 1982, 2002; US Department of Agriculture, 2009), South Dakota (South Dakota Game, Fish and Parks, 2008), south-central New Mexico (US Department of Agriculture, 2009) and Saskatchewan (Saskatchewan Ministry of Environment, 2008).

Currently, bison and elk within the greater Yellowstone area represent the only known sustainable reservoir of bovine brucellosis in wild species in the USA (Godfroid, 2002). Detection of the pathogen within wild animal populations raises concerns about transmission to domestic livestock. The brucellosis card test (along with standard plate agglutination, complement fixation, and rivanol testing) is an official serologic assay for detection of brucellosis in cervidae (US Department of Agriculture, 2003). However, because of the low sensitivity (80–94%) of the card test in experimental elk infections, the card test alone has a limited ability to diagnose brucellosis in individual animals (Thorne et al., 1978; Morton et al., 1981). No single serologic test should be used to diagnose brucellosis in elk; rather serologic tests should be combined or used in conjunction with bacterial culture of tissues (Thorne et al., 1978).

The potential for movement of disease agents during translocation of free-ranging

elk or other wildlife is discussed by Corn and Nettles (2001). Measures to protect against introduction of disease agents should be included in any translocation program. The regulated annual harvest of elk in Arkansas and Kentucky as part of a long-term management strategy presented the unique opportunity to evaluate the health of two reintroduced elk populations at different intervals posttranslocation (Arkansas, 13–25 yr; Kentucky 0–7 yr). However, due to constraints on the number of elk available and the selection biases that are inherent in this type of sampling, only tentative interpretations of disease absence and estimates of disease prevalence could be made. Despite the limitations described, we did not find evidence that disease agents had become established as a result of the introduction of free-ranging elk moved from Colorado and Nebraska to Arkansas from 1981 to 1985, or by introductions of elk from Arizona, Kansas, North Dakota, New Mexico, Oregon, and Utah to Kentucky from 1997 to 2002. The most significant disease relationship found in our surveys was infection by *P. tenuis*, an endemic nematode of the southeastern USA.

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